

# **EXHIBIT 6**

## T Cell Responses to Viral Infections

### *Lessons from Lymphocytic Choriomeningitis Virus*

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#### Abstract

The elaboration of a successful immune response is critical for the clearance of viral infections. CD8 T cells can directly kill virus-infected cells and also produce cytokines that modulate virus replication. Thus, the failure to induce or sustain these responses can profoundly impact the outcome of infections. Lymphocytic choriomeningitis virus (LCMV) infection of mice has proven to be one of the most informative experimental systems for examining antiviral T cell responses. In recent years, the application of newly developed approaches to analyze these responses has revealed that acute infections induce remarkably high levels of antiviral T cells. By contrast, protracted or chronic infections are associated with both the functional impairment and deletion of virus-specific CD8 T cells. This article discusses some of our findings using LCMV infection of mice as well as their relevance to other infections of animals and humans.

#### Key Words

T cells  
Viral infection  
Tetramers  
Cytokines  
Persistence

#### Introduction

Viruses are obligate intracellular parasites and consequently need to infect susceptible host cells in order to replicate. The host reacts to such an infection by mobilizing its immune system, and it is the elaboration of this response that controls the pathogen. The overall immune response comprises both innate (nonadaptive) components including interferons and natural killer cells, and antigen-specific (adaptive)

responses such as antibodies and T cells (1). The host's failure to induce or sustain an appropriate immune response, or the ability of the virus to evade this response, can have profound effects on the outcome of infection. There are numerous examples of viral infections that are not completely eliminated but are instead brought under control at a steady-state level (2). In this article, we discuss the role of T cells in controlling viral pathogens and highlight how the study of lymphocytic chorio-

meningitis virus (LCMV) infection of mice has demonstrated many key features of these responses.

CD8 T cells are potent effectors of the adaptive immune response and function either to directly kill virus-infected cells or to produce cytokines that purge replicating virus from infected cells (1,3,4). Consequently, CD8 T cell responses contribute significantly to the control of many virus infections. For virus-specific CD8 T cells to be effective, they must fulfill several requirements, including the following: they must be thymically selected and present in the periphery, be able to recognize and respond to infected cells, traffic to sites of infection, elaborate the appropriate effector functions necessary to resolve the infection, and sustain this effector activity until the infection is cleared. It is also desirable that, after the infection is resolved, a pool of virus-specific memory cells be established that confers immunity to reexposure to the same virus. Overall, the clearance of a viral infection represents an immunologic success story; the virus is eliminated and the host is now better able to control a second infection with the same pathogen.

Not all infections are rapidly cleared. The failure to control a viral infection can result from the emergence of viral variants that can escape immunosurveillance, the induction of weak primary immune responses, or the inability to sustain the immune response (1-3). Considerable correlative evidence supports the notion that weak CD8 T cell responses favor virus persistence. As discussed later, the impact of failing to sustain appropriate CD8 T cell effector activities is well exemplified by our work showing that during chronic LCMV infection, antigen-specific CD8 T cells become undetectable and/or fail to maintain their antiviral effector functions (5,6). Although informative studies regarding the regulation of T cell responses during chronic viral infections are being performed by numerous investiga-

tors, determining why cellular immune responses are ineffective at controlling certain viral infections remains an important challenge. It is likely that much of our understanding of how T cell responses are induced and regulated during chronic infections will continue to be determined using animal models.

LCMV is a natural mouse pathogen and a prototypic member of the Arenaviridae. Although LCMV is a relatively simple virus, encoding only four gene products, it has proven to be one of the best experimental systems for analyzing cellular immune responses. Studies on the immune response to this virus have been extremely informative, providing a foundation for our understanding of many fundamental immunologic concepts including major histocompatibility complex (MHC) restriction, tolerance, cytotoxic T lymphocyte activity, and immunologic memory (7-9).

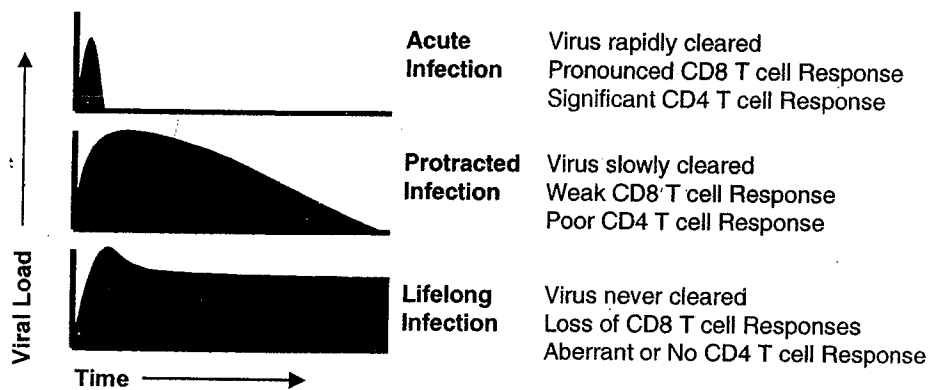
### Patterns of LCMV Infection

LCMV infection of mice can result in different outcomes, depending on the strain of virus used, the age of the host, and the route of infection. These range from the establishment of a lifelong carrier state that develops as a consequence of *in utero* infection, inoculation of neonatal mice, or infection of certain strains of immunodeficient mice, to a lethal meningitis that can occur following intracranial inoculation of normal adult mice. The various outcomes of LCMV infection reflect differences in the elaboration, activity, and localization of antiviral CD8 T cell responses. These responses are critical for viral clearance but can also mediate lethal immunopathology. Notably, if ineffective responses are elicited or a T cell tolerance state is created, then virus clearance may be compromised (7-9).

Inoculation of adult mice with LCMV by intraperitoneal (i.p.) or intravenous (i.v.) routes can result in an acute, protracted, or lifelong

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**Fig. 1.** Patterns of LCMV infection in adult mice. Peripheral infection of adult mice with LCMV results in different patterns of infection, depending on the strain of virus used and the immunocompetence of the host.

chronic infection, and this is determined by the strain of virus used and the genetic background of the host (Fig. 1). By examining immune responses elicited during these different types of infections, we are currently defining the properties of antiviral immune responses that rapidly resolve acute infections, only slowly control more protracted infections, or fail to control lifelong chronic infections.

### Measuring Virus-Specific T Cell Responses

In recent years, the development of novel approaches to analyze T cells at the single-cell level has advanced our understanding of how CD8 T cell responses are induced and regulated. The use of MHC class I tetramers to enumerate the physical presence of antigen-specific CD8 T cells and the measurement of cytokine-producing cells by either enzyme-linked immunospot (ELISPOT) assays or intracellular staining have revealed that pronounced virus-specific CD8 T cell responses are elicited during the acute phase of many viral infections, including LCMV (10,11). Before these techniques were available, most assays for CD8 T cells relied on measuring the activity of bulk populations of cells. Tech-

niques such as radiochromium release assays, tritiated thymidine incorporation, and quantitation of cytokines in stimulated cell culture supernatants provide important information, but these approaches also have limitations (12). They usually require that the responding cell population be expanded in vitro before any activity is measured. Moreover, since these assays rely on a functional readout, they do not determine whether antigen-specific cells are present which fail to register in the assay because they do not exhibit the particular activity being scrutinized. Another and arguably more severe drawback is that these functional assays do not provide accurate information regarding the actual number of antigen-specific cells present. Although limiting dilution analysis can be employed to quantitate the actual numbers of antigen-specific cells, this approach is also restricted by the limitations just mentioned. In addition, these assays are cumbersome to set up and, in many instances, are thought to underestimate the actual frequency of antigen-specific cells.

In 1996, Altman et al. (13) reported the development and use of tetrameric MHC class I complexes to detect and enumerate human immunodeficiency virus (HIV)-specific CD8 T cells. At that time, it was clear

that the application and use of MHC tetramer technology would advance our understanding of CD8 T cells and antiviral immunity. MHC class I tetramers can be used in a similar manner to fluorescent antibodies, but these reagents bind to and identify all T cells that express T cell receptors (TCRs) that recognize a specific MHC class I-peptide combination. MHC class I tetramers are used to directly visualize antigen-specific CD8 T cells, and this can be applied to define the pattern of cell-surface marker expression by the T cell population of interest. In addition, these reagents can be used to both isolate and activate antigen-specific cells.

An advantage of MHC class I tetramer technology is that no functional readout is required in order to enumerate the T cell population of interest. Nevertheless, this also represents a drawback because no information is obtained regarding its biologic activity. Although cytotoxicity is a major effector activity of CD8 T cells, it is difficult to determine the actual number of cytotoxic antigen-specific cells within a bulk population. CD8 T cells, however, also produce cytokines including interferon- $\gamma$  (IFN- $\gamma$ ), tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-2 (IL-2). Thus, it is possible to quantitate the numbers of CD8 T cells that are capable of producing the cytokine of interest. ELISPOT assays and intracellular staining have been employed to enumerate cytokine-producing cells. In these assays, the responder cells are briefly restimulated in vitro usually with a synthetic peptide epitope. In this way the number of cytokine-producing cells specific for the chosen peptide epitope can be determined (3,5,10,11).

### Acute Infections

The use of both MHC class I tetramer staining and assays to enumerate cytokine-

producing cells have revealed that acute LCMV infection induces massive expansion and activation of virus-specific CD8 T cells (10,11). The kinetics of the LCMV-specific CD8 T cell response during acute infection is shown in Fig. 2A and can be broken down into three distinct phases: activation and expansion, contraction, and memory. The activation and expansion phase occurs during the first week of infection. During this period, virus-specific CD8 T cells proliferate vigorously, and there is approximately a 10,000-fold expansion in the number of virus-specific CD8 T cells. By 8 days after infection, at the peak of the response, 50% of splenic CD8 T cells have been shown to be LCMV specific (11). These virus-specific cells are potent effectors and exhibit direct ex vivo cytotoxicity as well as produce cytokines including IFN- $\gamma$ . This overwhelming response purges the host of virus-infected cells. After replicating virus is cleared, a contraction phase ensues during which >90% of the virus-specific T cells undergo apoptosis. A stable pool of memory cells is then established as homeostasis is restored. These memory cells mount anamnestic responses if the host becomes reexposed to LCMV and function to control more rapidly the rechallenge inoculum. This recall response can protect against intracranial rechallenge by resolving the infection before lethal immunopathology can occur (7-9).

The virus-specific CD8 T cells that respond during acute LCMV infection are not a monoclonal population of cells with a single specificity. Instead, this response comprises oligoclonal subsets of cells that recognize various distinct viral epitopes. This is well exemplified in H-2<sup>b</sup> mice, which mount responses to at least six distinct viral epitopes (11,14-16). Since the magnitude of the response to each individual epitope is not equal, a hierarchy of immunodominant and subdominant responses

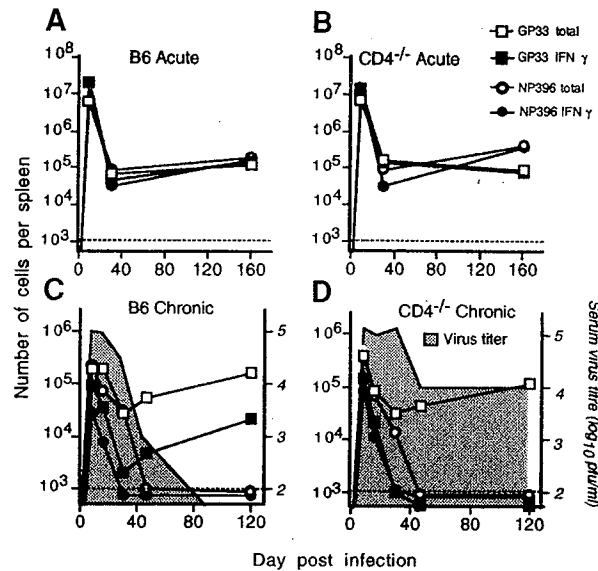
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**Fig. 2.** Kinetics of LCMV-specific CD8 T cell responses during acute, protracted, and chronic infections. Splenocytes were prepared from normal C57BL/6 mice and CD4<sup>-/-</sup> mice at various days after acute infection with LCMV-Armstrong (**A,B**) or during protracted infection with LCMV-clone 13 (**C,D**). The total number of GP33-41 (□) and NP396-404 (○)-specific CD8 T cells was enumerated by MHC tetramer staining. Intracellular cytokine staining or ELISPOT assays for IFN-γ production was performed to measure the functional activity of these cells (●,■). Serum virus titers are below the limit of detection in acutely infected animals but are represented by the gray area in clone 13-infected mice. Mean values are shown for two to six mice at each time point. (Panels [C] and [D] reproduced from ref. 5 by copyright permission of The Rockefeller University Press.)

emerges. This hierarchy is apparent at the peak of the response and this order of dominance remains similar during the contraction and memory phases following acute infection. Figure 3 shows the patterns of LCMV-specific CD8-T cell responses in H-2<sup>b</sup> (C57BL/6) mice visualized by both intracellular cytokine analysis and tetramer staining at 83 d post acute infection. These data illustrate several points regarding LCMV-specific memory CD8 T cells in immune mice. First, MHC class I tetramers can identify the physical presence of memory CD8 T cells. Second, these memory cells express elevated levels of the cell-surface marker CD44. Third, responses to several viral epitopes are detectable. Fourth, a distinct hierarchy of responses is apparent. Fifth, memory CD8 T cells do not constitu-

tively synthesize cytokines but produce both IFN-γ and TNF-α if briefly restimulated in vitro with synthetic peptide epitopes. Finally, there is a good correlation between the numbers of CD8 T cells visualized by tetramer staining and those that produce cytokines as detected by intracellular staining. Curiously, these data also show that more CD8 T cells appear to be producing IFN-γ and TNF-α in response to stimulation with the GP33-41 peptide epitope than are detectable by staining with the H-2D<sup>b</sup>(GP33-41) tetramer. This discrepancy may be because the GP33-43 region encodes two overlapping H-2<sup>b</sup>-restricted epitopes, an H-2D<sup>b</sup>-restricted GP33-41 epitope, and an H-2K<sup>b</sup>-restricted GP34-41 epitope (15,17). The differences between the magnitude of the response detected by H-2D<sup>b</sup>(GP33-

41) tetramer staining and those revealed by intracellular cytokine analysis most likely occur because the MHC class I tetramer used only detects the H-2D<sup>b</sup>-restricted GP33-41-specific CD8 T cells, whereas stimulation with the GP33-41 peptide activates both the H-2D<sup>b</sup>- and H-2K<sup>b</sup>-restricted responses.

Pronounced expansion of virus-specific CD8 T cells is not unique to acute LCMV infection of mice; parallel observations have been made during the acute phase of other viral infections. Studies of viral infections of mice have shown that sizable expansions of virus-specific CD8 T cells occur following infection with numerous pathogens including influenza, respiratory syncytial virus, and murine-gammaherpes virus 68 (18–21). Analyses of virus-specific CD8 T cell responses in humans mirror findings reported in animal models. Utilization of MHC tetramer technology to measure frequencies of CD8 T cell responses to Epstein-Barr virus (EBV) antigens in patients with recent-onset acute infectious mononucleosis has shown that up to 44% of circulating CD8 T cells can be specific for a single lytic protein epitope (22). Although the frequency declines following resolution of symptoms, memory responses to the same epitopes are readily detectable in long-term healthy virus carriers. The maintenance of these virus-specific CD8 T cells plays a central role in ensuring an asymptomatic virus-host equilibrium (23). This observation is supported by clinical evidence of increased incidence of virus-mediated lymphoproliferative disease in allograft recipients receiving immunosuppressive therapy, and the occurrence of disease remission following adoptive transfer of in vitro-expanded autologous EBV-specific CD8 T cells (24,25). Oligoclonal expansions of CD8 T cells have also been reported during the acute phase of HIV infection, and tetramer analysis has demonstrated that these CD8 T cells are virus specific

(26,27). The importance of CD8 T cells in the initial control of lentiviral infections has been highlighted by CD8 T cell depletion studies carried out in simian immunodeficiency virus (SIV)-infected rhesus monkeys. Ablation of CD8 T cells by antibody treatment is associated with diminished control of SIV replication and faster disease progression (28,29). Virus-driven expansion of antigen-specific CD8 T cells has been documented during hepatitis C virus (HCV) infection, and measurable responses against HCV epitopes derived from the viral nonstructural proteins NS3 and NS5 have been detected (30,31).

Although acute LCMV infection is characterized by an overwhelming virus-specific CD8 T cell response, a sizable virus-specific CD4 T cell response is also induced (16, 32–34). Virus-specific CD4 T cell responses are readily detectable by both intracellular cytokine staining and ELISPOT assays. These CD4 T cells primarily produce IL-2 and other Th1-associated cytokines, although production of Th2-associated cytokines has been detected in certain instances. In our studies following acute infection, generally similar patterns of CD8 T cell responses were detectable in CD4 T cell deficient (CD4<sup>-/-</sup>) mice or in mice depleted of CD4 T cells by antibody treatment (Fig. 2B). This suggests that, if the infection is rapidly resolved, CD4 T cells are dispensable for the induction and maintenance of CD8 T cell activity. This finding is in contrast to one report showing that CD8 T cell memory fades in the absence of CD4 T cells following acute LCMV infection (35). Overall, it is likely that, in the normal host, CD4 T cell responses assist the proliferation and activity of CD8 T cells and also help the elaboration of antiviral antibody responses (36).

### Protracted and Chronic Infections

Many viral infections are not rapidly cleared and are either brought under control more

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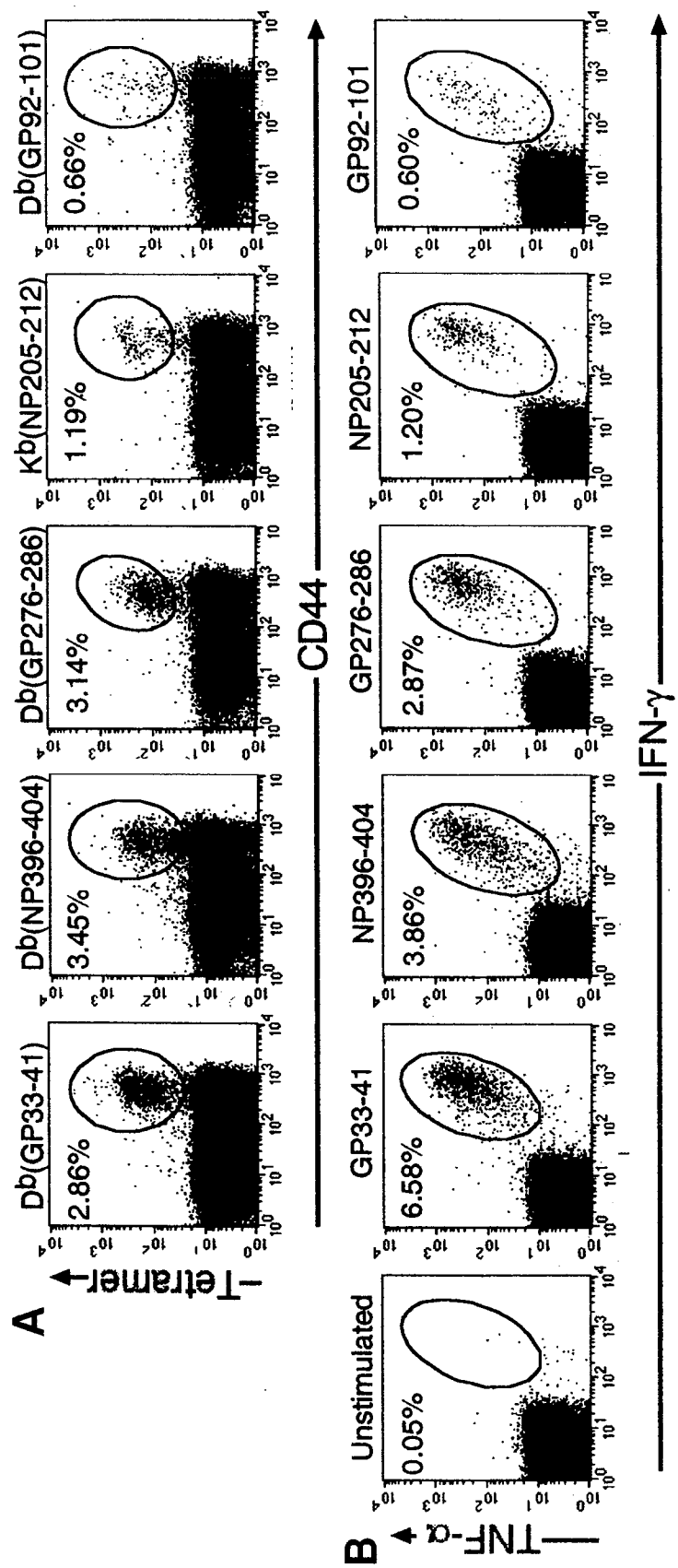
slowly or persist for the life of the host (2). In adult mice a protracted infection ensues following inoculation with high doses of certain strains of LCMV (Fig. 1). These viral isolates typically contain a phenylalanine-to-leucine substitution at amino acid residue 260 of the viral glycoprotein that enhances affinity of the viral glycoprotein (GP) to the cellular receptor  $\alpha$ -dystroglycan (37–40). The viral isolates aggressively infect dendritic cells within the splenic white pulp; however, these infections are not restricted to the spleen and become widely disseminated, affecting many tissues and organs throughout the mouse (40).

The pattern of slow viral clearance that occurs during protracted LCMV infection suggests that, under these conditions, the virus-specific CD8 T cell response is ineffective. In our laboratory, we are investigating the induction, regulation, and activity of virus-specific T cells following inoculation of adult mice with the clone 13 strain of LCMV (Fig. 2C) (5,6). During the first week following infection, a high-grade ( $10^4$ – $10^5$  plaque-forming units/mL of serum) viremia becomes detectable and is subsequently slowly resolved over a period of several months. Even though the infection is not rapidly controlled, a virus-specific CD8 T cell response is initially induced, and by 1 week after inoculation, this response can be detected by both MHC class I tetramer staining and ELISPOT or intracellular staining for cytokine production (5). This pool of virus-specific CD8 T cells recognizes multiple viral epitopes derived from both the viral GP and viral nucleoprotein (NP) (unpublished observation). By comparison with the virus-specific CD8 T cell responses elicited by acute LCMV infection, during protracted LCMV infection the overall magnitude of the response is lower, the effector activity is impaired, and the epitope hierarchy is altered.

During protracted and chronic LCMV infection of adult mice, virus-specific CD8 T cells

succumb to different fates depending on the viral epitope recognized. Unlike acute LCMV infection, in which there is a good correlation between the numbers of virus-specific CD8 T cells visualized by MHC class I tetramer staining and those that produce IFN- $\gamma$ , effector activity cannot be ascribed to all of the virus-specific CD8 T cells that are detectable during the course of protracted LCMV infection (Figs. 2 and 3) (5,41). Analysis of CD8 T cells specific for the H-2D<sup>b</sup>-restricted GP33 epitope has revealed that although these cells are physically present in the spleen of the protractedly infected host, many of these cells lose the capacity to elaborate any detectable antiviral effector activity, including cytotoxicity and cytokine production. Similarly, during protracted LCMV infection, not all of the CD8 T cells detectable using H-2D<sup>b</sup> NP396-specific tetramers remain functional. In contrast with H-2D<sup>b</sup>-restricted GP33-specific CD8 T cells that are maintained in this effector function-negative state, the H-2D<sup>b</sup>-restricted NP396-specific T cells become undetectable by tetramer staining. It is unclear why this dichotomy between unresponsiveness and deletion emerges. CD8 T cell responses are usually antigen driven, and it is possible that the degree of activation determines whether the responding cells are deleted or become functionally silenced. The viral NP is the most abundant viral protein present within infected cells, and CD8 T cells that recognize epitopes derived from this protein may first become unresponsive and then succumb to deletion, whereas those that recognize GP-derived epitopes lose effector activity but do not progress to deletion (5,7–9). Thus, depending on the degree of antigenic stimulation, diminished CD8 T cell responses during protracted LCMV infection result from either the functional impairment of the virus-specific cells or their physical loss from the host. Nevertheless, in normal mice, sufficient virus-specific CD8





**Fig. 3.** LCMV-specific CD8 T-cell responses in immune mice. Splenocytes from LCMV-Armstrong-infected C57BL/6 mice were prepared 83 days after inoculation. **(A)** The physical presence of virus-specific CD8 T-cells was analyzed by staining with MHC class I tetramers complexed with five separate viral epitopes. **(B)** IFN- $\gamma$  and TNF- $\alpha$  production by LCMV-specific CD8 T cells was determined by intracellular cytokine staining following in vitro stimulation with the indicated peptide epitopes. Gated CD8 T cells are shown, and the values given in each plot represent the percentage of CD8 T cells that either stained with the indicated tetramer or produced TNF- $\alpha$  and IFN- $\gamma$ .

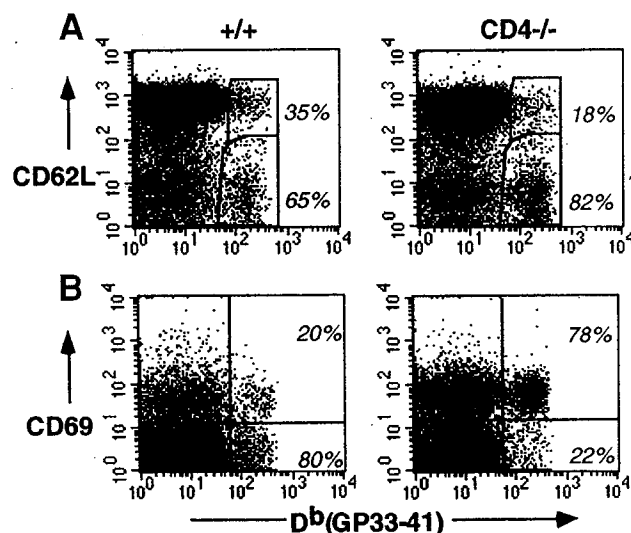
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T cells remain functionally active to keep the infection in check and eventually resolve it.

A more pronounced silencing of virus-specific CD8 T cells occurs in the absence of CD4 T cells. A lifelong carrier state is established following infection of adult CD4<sup>-/-</sup> mice with LCMV clone 13 (Fig. 2D) (5). Under these conditions, virus persistence is associated with the complete loss of virus-specific CD8 T cell activity. Initially, the CD8 T cell response in CD4<sup>-/-</sup> mice resembles that in normal mice infected with LCMV-clone 13 (Fig. 2C). Responses are detectable by both MHC class I tetramer staining and single cell assays for cytokine production; however, unlike acute infection, not all of the CD8 T cells visualized by tetramer staining can be accounted for by functional assays. After the initial induction of the response, NP396-specific CD8 T cells become undetectable by both functional assays for IFN- $\gamma$  production and by MHC class I tetramer staining. The H-2D<sup>b</sup>-restricted GP33-specific T cells lose effector activity but are maintained for prolonged periods in vivo in this effector function-negative state (Fig. 2D) (5). Examination of activation marker expression by these unresponsive CD8 T cells has shown that even though these cells fail to elicit effector activity they retain an activated cell surface phenotype. They are predominately low in CD62L but express high levels of CD43 (IBII) and CD69 (Fig. 4) (unpublished observations) (5). Therefore, these effector function-negative CD8 T cells appear to be engaged in the Sisyphean task of responding to the viral antigen but fail to accomplish their goal of elaborating downstream effector activities. The biologic consequence of this is failure to control the infection.

Studies of protracted and lifelong LCMV infection of mice have shown that, under conditions of high antigen load and weak CD4 T cell responses, virus-specific CD8 T cell

activity is diminished. However, the precise mechanism that impairs the effectiveness of this response varies depending on the epitope recognized. CD8 T cells specific for certain epitopes persist in an activated but functionally silenced state whereas T cells specific for other epitopes become deleted. Loss of CD8 T cell activity does not, however, occur only during persistent LCMV infection. One of the best-studied human viruses is HIV, and analysis of immune responses to this virus have helped identify a number of factors that can result in the failure to control chronic infections. Perturbations in the host immune response to HIV include high dose tolerance, clonal exhaustion, shifts in viral epitope specificity, and skewed maturation of the memory CD8 T cell response (42–44). An analysis of the TCR repertoire of virus-specific CD8 T cells has demonstrated a rapid disappearance of a significant number of virus-specific T cell clones involved in the primary response. Moreover, examination of the epitope specificity of CD8 T cells in chronically infected HIV patients indicates that responses that may dominate during the later stages of infection can differ significantly from those present during the acute phase. Thus, the deletion of certain virus-specific CD8 T cells may contribute to virus persistence and disease progression. In addition to the physical loss of virus-specific CD8 T cells, the functional impairment of virus-specific CD8 T cells may also affect the ability of the host to control chronic infections. Such impairment may include selective defects in cytokine production and/or cytotoxic function. Effector function deficits have been described both in animal models of uncontrolled, persistent viral infection, such as mouse polyoma virus, and in clinical observations in patients infected with HCV or with progressive HIV disease (31,45–49). Moreover, effector function-negative, tumor-



**Fig. 4.** Effector function-negative CD8 T cells are phenotypically activated. Splenocytes were prepared from C57BL/6 mice (+/+) and CD4<sup>-/-</sup> mice 108 days after infection with LCMV-t1b. Preparations were stained with anti-CD8 antibodies and H-2D<sup>b</sup>(GP33-41) tetramers together with either (A) anti-CD62L or (B) anti-CD69 antibodies. Gated CD8 T cells are shown, and the values represent the percentage of GP33-41-specific CD8 T cells present within the regions indicated. Note that infection of adult CD4<sup>-/-</sup> mice with LCMV-t1b results in virus persistence and the loss of effector activity by GP33-41-specific CD8 T cells.

associated antigen-specific CD8 T cells have also been reported in a patient with metastatic melanoma (50). Although the precise extent and biologic consequence of such functional impairments during chronic infections of humans are not fully understood, it is likely that viral infections are more successfully controlled by robust, functionally competent CD8 T cell responses.

Our studies of CD4<sup>-/-</sup> mice have revealed that CD4 T cell responses also contribute to the control of chronic viral infections by sustaining CD8 T cell effector functions (5,51). Studies in HIV-infected individuals suggest that the targeted destruction of CD4 T cells by the virus can adversely influence the long-term maintenance and quality of the virus-specific cytotoxic CD8 T cell response (52–54). This is supported by the observation that early therapeutic intervention, which reduces infection of CD4 T cells, is associated with preservation of CD4 T cell activity and the maintenance of effective virus-specific cytotoxic

CD8 T cell responses (55). The potential importance of CD4 T cell responses is further suggested by results from clinical trials of adoptive immunotherapy using cytomegalovirus (CMV)-specific cytotoxic CD8 T cells in allogeneic bone marrow recipients. Effective reconstitution of cellular immunity against CMV was demonstrated only in recipients possessing adequate CMV-specific CD4 T cells (56). In addition to their “helper” roles, CD4 T cells can elicit more direct antiviral effector activities including the production of cytokines and the induction of target cell lysis (36). Taken together, experimental data from numerous systems demonstrate that CD8 and CD4 T cell responses cooperate to control viral infections (51).

## Conclusion

LCMV infection of mice continues to be a convenient and informative system to dissect the parameters that modulate antiviral immune

responses. The application of recent technological advances to measure T cell responses, including MHC class I tetramer staining and single-cell assays for cytokine production, has revealed that acute LCMV infection induces a massive antigen-specific CD8 T cell response. By contrast, analysis of protracted and chronic LCMV infections has shown that under these conditions CD8 T cell responses are induced but become immunologically silenced. In immunocompetent hosts, this unresponsive phenotype is not absolute and sufficient effector activity is retained to resolve the infection slowly. The immunologic silencing of the virus-specific CD8 T cell response

is more pronounced in CD4-deficient hosts, highlighting the role of CD4 T cell responses in sustaining CD8 T cell effector activity under conditions of persistent antigenic stimulation. Overall, these findings illustrate that observations in the LCMV system provide a useful platform for the comparative analysis of cellular immunity induced by other viral infections as well as tumors.

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